bio-protocol Preprint

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Skin penetration studies

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An abbreviated version of this protocol was published in Science Advances in Jul 2020

Treatment of psoriasis with NFKBIZ siRNA using topical ionic liquid formulations

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Detailed protocol

Porcine skin studies were carried out in Franz diffusion cells (FDC) with penetration area of 1.77cm2 .
The porcine skin was obtained from Lampire Biological Laboratories, Pipersville, PA, USA. Briefly skins were thawed, hairs were trimmed, and washed with phosphate buffer saline (PBS, pH 7.4).
A 36mm punch was used to cut out a disc of the skin and a scalpel was used to get rid of the connective tissues and subcutaneous fat layers.
The skin (roughly 0.5mm thick) was placed on the diffusion cell with the stratum corneum (SC) layer facing upwards.
The acceptor component of the cell was filled with PBS (~12mL) and equipped with a magnetic stirrer bar.
1mL PBS was added to the donor chamber and the conductivity was measured using a waveform generator (Agilent 33120) and voltmeter (Fluke 87 True RMS Multimeter) at a frequency of 100 Hz and amplitude,100 mV. Only skin samples with a measured transepidermal conductivity of less than 10 μA were used for further studies.
The cells were kept in an oven at 37ºC to warm up.
The donor compartment was left for 5mins before applying 20μL of Cy5-labelled siRNA-IL (siRNA 50μM) solution on top of the skin ensuring full coverage.
The donor chamber and side-arm of the cells were sealed with parafilm/foil and eppendorf respectively to reduce evaporation and were incubated at 37 °C for 24 h on a stirrer plate.
Following incubation, the skin was removed from the cell, washed gently with PBS and were further analyzed using tape-stripping and confocal microscopy.
After removal of the skin from the cell and washing in PBS, the SC was stripped from the epidermis using an adhesive tape up to ten layers (SC1, SC2-5, SC6-10).
Following SC removal, the epidermis was separated from the dermis using a surgical sterile scalpel and a third of dermis (by area) was removed by punching with 4mm three times.
Each layer was collected separately in glass vials containing 1mL of PBS/methanol (1:1) mixture and was left to shake overnight to extract the Cy5-siRNA from the skin layers, which was further analyzed using a plate reader (Tecan Safire, AG, Switzerland) on a 96-well plate at an excitation wavelength of 633 nm and emission wavelength of 665 nm.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Mandal, A. (2020). Skin penetration studies. Bio-protocol Preprint. bio-protocol.org/prep635.
2. Mandal, A., Kumbhojkar, N., Reilly, C., Dharamdasani, V., Ukidve, A., Ingber, D. E. and Mitragotri, S.(2020). Treatment of psoriasis with NFKBIZ siRNA using topical ionic liquid formulations . Science Advances 6(30). DOI: 10.1126/sciadv.abb6049